Persistent atrial fibrillation in a goat model of chronic left atrial overload

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Objectives: Atrial dilatation predisposes to atrial fibrillation. Although several animal models focus on the initiating mechanisms of atrial fibrillation in dilated atria, a model of left atrial overload resulting in persistent atrial fibrillation in nonanesthetized animals has not been presented thus far.

Methods: In 24 goats a vascular shunt was implanted between the aorta and the left atrium through a left thoracotomy. In 6 animals the shunt was ligated immediately (control group). Ultrasonic crystals were implanted to monitor atrial dilatation. Bipolar electrodes were positioned epicardially on the left atrium for measurement of the atrial effective refractory period, conduction times, and atrial fibrillation duration.

Results: Four weeks of overload resulted in an increase of left atrial pressure (23.1 ± 6.8 mm Hg in the open-shunt group vs 7.0 ± 1.9 mm Hg in the control group, $P = .002$) and a progressive dilatation of the left atrium (135% ± 20% in the open-shunt group vs 98% ± 8.0% in the control group, $P = .002$). Among the open-shunt group's long-term survivors (n = 12), 9 animals showed prolonged atrial fibrillation (>1 hour), and of these, 6 were in persistent atrial fibrillation (>1 week). The atrial effective refractory period increased during the first week and remained prolonged until death (182 ± 11 ms in the open-shunt group vs 161 ± 15 ms, $P = .03$). The conduction time did not change. An increase in collagen formation was noticed in both groups, without a significant difference between them.

Conclusions: A chronic aortic to left atrial shunt is a feasible model in the goat. It induces progressive left atrial dilatation with an increased atrial fibrillation duration up to hours in the majority of animals. Prolonged atrial fibrillation duration could not be explained by a shortening of atrial effective refractory period or increase in fibrosis.

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Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia and leads to considerable morbidity and mortality.[1] Experimental and clinical studies have shown that left atrial (LA) dilatation predisposes to AF.[2-4]

Acute and chronic animal models have been developed to elucidate the correlation between atrial dilatation and AF. A common finding of the acute experiments is that on abrupt atrial stretching, AF vulnerability increased.[5-7] The majority of the chronic dilatation models focused on the initiating mechanisms of AF in dilated atria. In a dog model of chronic LA overload induced by chordal rupture, increased vulnerability to AF is noticed in the absence of wavelength decrease and in the presence of interstitial fibrosis.[8] A second dilatation model induced by His-bundle ablation shows short AF paroxysms and local conduction delays without evident atrial fibrosis.[9] Although these models give us a better insight into the correlation between atrial dilatation and AF, an LA dilatation model of persistent AF in nonanesthetized animals is yet to be described.[8-13]

The aim of the current study was to develop a model of chronic overload in the goat induced by a vascular shunt between the aorta and the LA. Progression of atrial dilatation and fibrillation was studied over several weeks in nonanesthetized animals.

Abbreviations and Acronyms
AERP = atrial effective refractory period
AF = atrial fibrillation
LA = left atrium

Materials and Methods

Study Animals and Surgical Procedure

Twenty-four goats were randomly assigned to an open-shunt (n = 18) or a control (n = 6) group. Body weight was 68.0 ± 8.3 kg. Animal handling was in accordance with the "Guide for the care and use of laboratory animals" (published by the US National Institutes of Health) and approved by the Animal Investigation Committee of the University of Maastricht.

General anesthesia was induced with 20 mg/kg thiopental administered intravenously. The animals were ventilated with isoflurane (2%) and a 1:2 mixture of O_2 and N_2O. Oxygen saturation, electrocardiography, and rectal temperature were monitored. Arterial pressure was measured continuously. Fluid loss was compensated (500 mL/h Ringer's lactate). Antibiotic prophylactics consisted of 20 mg/kg ampicillin administered intravenously and repeated after 4 hours, and 3 mg/kg gentamicin was administered once intravenously. Data were stored with a 32-channel acquisition system (IdeeQ 1.70; Instrument Development Engineering and Evaluation, University of Maastricht, The Netherlands).

Animals underwent a left thoracotomy. The pericardium was opened, and an LA pressure catheter (Leycom, 6Fr) was inserted. The LA was chronically instrumented with piezo-electric crystals positioned circularly around the lateral atrial wall (Sonometrics, London, Canada). Two silicon patches, each with 2 silver bipolar electrodes (1 mm in diameter; interelectrode distance, 5mm), were positioned on the LA lateral wall and fixed to each other (interpatch distance, 3 cm). After heparinization (100 U unfractioned heparin per kilogram of body weight), successive site clamping of the aortic arch and the LA was performed to interpose a polytetrafluorethylene (Gore-Tex; W. L. Gore, Inc, Newark, Del) 8-mm shunt. The choice of 8 mm in diameter was based on pilot studies. The shunt was opened progressively. In the control group the shunt was ligated immediately (shunt time, 4.6 ± 1.1 minutes).

At the end of the procedure, all catheters were removed. Electrode leads and crystals were exteriorized to the neck. Analgesic treatment consisted of 0.05 mg/kg buprenorphine administered intramuscularly up to 3 times a day. Anticoagulant treatment included 75 mg of clopidogrel administered orally and 11,400 International Units (IU) of nadroparin administered subcutaneously once a day. During a postoperative monitoring period, all animals were regularly subjected to physical examination.

Atrial Overload Quantification

Mean LA pressure was measured directly in the atrial cavity during temporary ventilatory arrest. A preshunt value was recorded after opening the pericardium. Acute hemodynamic overload was registered 30 minutes after shunt interposition. Chronic overload was evaluated before death, at which time a small left thoracotomy was performed after achievement of general anesthesia, and LA pressure was measured by means of direct puncture. Invasive mean arterial pressure was recorded in a similar time protocol.

LA dilatation was evaluated in diastole. Distances between multiple pairs of opposing crystals were averaged. Preshunt values were measured immediately after crystal affixation. Acute volume overload was determined half an hour after shunt interposition. Chronic volume overload was evaluated weekly in nonanesthetized animals. LA enlargement was expressed as a percentage of preshunt values (100%).

Electrophysiologic Study

During surgical intervention, electrophysiologic studies were performed before and after opening the shunt. From the third postoperative day, chronic atrial overload was evaluated several times a week in nonanesthetized animals. Atrial effective refractory period (AERP) was measured on the LA epicardial wall. A train of 10 stimuli (S1) with a pacing interval of 400 ms, 4 times the threshold, was followed by a premature stimulus (S2). AERP was defined as the longest S1S2 interval that failed to produce a propagated response. Atrial conduction times were measured between both electrode patches (4× threshold, 400 ms pacing rate) with fixed interpatch distance. Several time intervals between pacing spikes and captured electrograms were averaged.

AF duration was registered as the mean of 10 AF responses after burst pacing (1 second, 50 Hz, 4× threshold) and as the maximal response during each measurement session. If AF episodes were longer than 1 hour, 5 instead of 10 AF episodes were averaged. Persistent AF was defined as an AF duration of 7 days or longer. The persistent AF subgroup was compared with the self-terminating subgroup with regard to LA pressure and dilatation to evaluate the correlation between LA overload and AF duration.

Atrial electrograms were registered by using the epicardial electrodes. Atrial fibrillation cycle length was computed after AF stabilization but within 10 minutes after its initiation. Intervals between the steepest negative deflections in a 1-minute period were measured, and the median value was calculated.

Histology

Animals were killed after 4 to 5 weeks. The heart was examined macroscopically, LA wall thickness was measured, and graft patency was evaluated. Multiple transmural tissue samples from different sites of the LA were fixed in 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, and sectioned in 4-μm-thick slices. Serial sections were stained with hematoxylin and eosin, periodic acid–Schiff for glycogen storage, or Masson's trichrome to assess the amount of connective tissue (Ventana Automated Stainer; Ventana Medical Systems, Tucson, Ariz). An experienced pathologist (C.G.) analyzed the microscopic images qualitatively. For quantitative analysis,[14] digitization was done with a Nikon Digital Sight DS-5M-L1 camera (Nikon Corp, Japan) associated with a Zeiss axiolab microscope (Carl Zeiss, Göttingen, Germany). Measurements were performed with the open-source software package ImageJ (developed by W. Rasband at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Images were converted in 256-gray values. A constant windows gray value was adopted for all cases before transformation to a bitmap image. The percentage of black pixels in each image was measured for the amount of glycogen[15] (100× magnification) and fibrosis (20× magnification).

Statistics

Data are presented as means ± standard deviation. The Mann–Whitney *U* test was used to compare the results between the open-shunt group and the control group at a specific moment in time. The Wilcoxon signed-ranks test was used to assess the evolution in time within a group. The R^2 value was calculated by using a transformed regression model to estimate the correlation between AF duration and LA dilatation.

Results

Feasibility of Shunt Construction

Shunt interposition was feasible in all goats, without operative deaths. A continuous murmur confirmed its patency. At death (28.6 ± 13.9 days), 2 animals showed shunt thrombosis. Heart rate (results not shown) and body weight did not change significantly before the operation (68.0 ± 8.3 kg) compared with at death (65.4 ± 9.0 kg).

Five animals died early because of early-onset pulmonary edema (n = 2), wound infection (n = 1), or profuse hemodynamic deterioration (n = 2). This resulted in an available study population of 12 animals in the open-shunt group and 5 animals in the control group.

LA Overload

In the open-shunt group there was an increase in mean LA pressure from 7.0 ± 2.2 mm Hg before the shunt to 14.6 ± 4.4 mm Hg ($P = .003$) 30 minutes after shunt construction and up to 23.1 ± 6.8 mm Hg ($P = .003$) at death (Figure 1). In the control group LA pressure did not change significantly. Differences between groups were significant after 30 minutes ($P = .02$) and at death ($P = .002$). Mean arterial blood pressure decreased in the open-shunt group from 111.1 ± 7.8 to 81.9 ± 14.0 mm Hg after opening the shunt ($P = .008$). At death, blood pressure returned to preshunt values (107.9 ± 11.2 mm Hg). LA size increased in the open-shunt group up to 112% ± 8% thirty minutes after opening the shunt and during chronic monitoring up to 135% ± 20% (Figure 2). Values were normalized to initial baseline values (baseline is 100%). LA enlargement was significant compared with that in the control group for each point in time.

AF Duration

Mean AF duration before shunt interposition was 0.09 ± 0.06 minutes. During chronic overload, spontaneous AF or other supraventricular arrhythmias were never observed. In the open-shunt group a progressive and significant increase in mean AF duration was demonstrated, starting from the first week of overload. After 4 weeks, mean AF duration was 4189 ± 4777 minutes in the open-shunt group versus 0.4 ± 0.3 minutes in the control group ($P = .02$). The maximal AF duration for each individual animal is plotted against time of overload in Figure 3. Persistent AF (>1 week) could be induced in 6 of 12 animals in the open-shunt group. In 3 of the remaining animals, the longest observed AF episodes passed 1 hour (110, 240, and 7200 minutes, respectively), and in 3 animals the maximal AF duration was only slightly increased (0.5, 2, and 15 minutes, respectively).

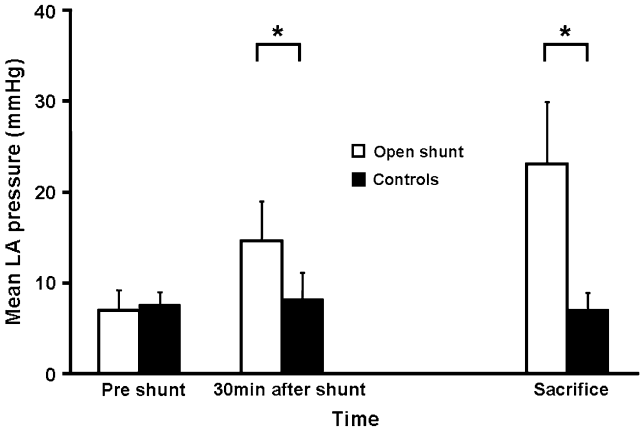

Figure 1. Mean left atrial (LA) pressure during surgical intervention and at death in the open-shunt and control groups. In the open-shunt group LA pressure increased significantly *(*)* after shunt activation and at death compared with that seen in the control group.

Relation Between LA Overload and AF

In Figure 4 all registered AF durations are plotted against their corresponding LA size. Short AF durations (<1 minute) were recorded, regardless of increase in LA size. By contrast, long AF durations (>100 minutes) were only obtained if LA segment length was increased to more than 110%. The correlation index between both parameters remained low

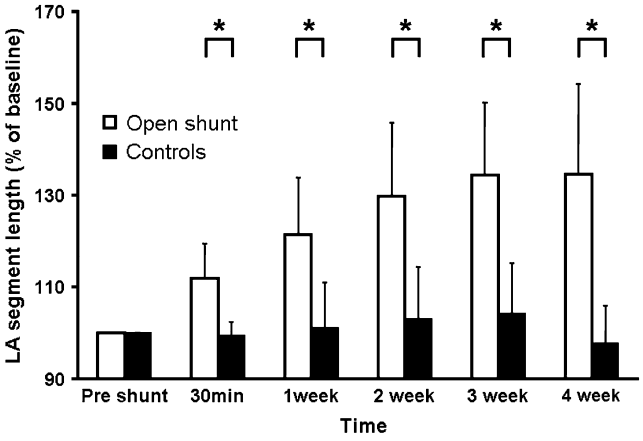

Figure 2. Operative and postoperative evolution of left atrial *(LA)* segment length equated to baseline in the open-shunt and control groups. LA segment length increased significantly *(*)* in the open-shunt group compared with that in the control group at the same moment in time.

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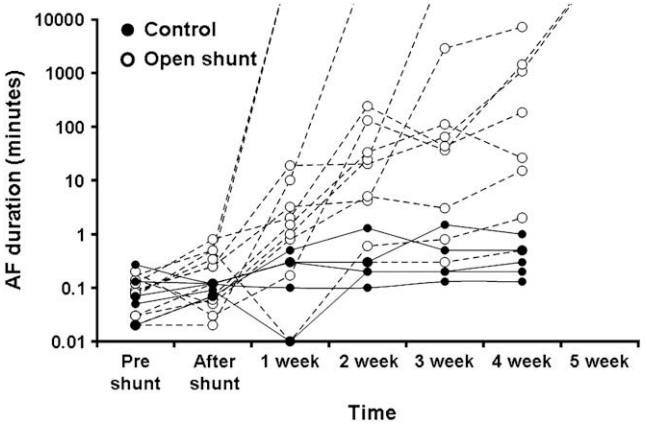

Figure 3. The maximal atrial fibrillation *(AF)* duration registered weekly in individual animals and plotted against time of overload. In the open-shunt group 6 animals presented with persistent AF. In another 3 animals AF lasted longer than 1 hour. In the control group AF duration evolved not significantly compared with preshunt values. One day represents 1440 minutes, and 1 week represents 10,080 minutes.

(R^2 = 0.03) because of the wide variation in AF durations given a specific dilatation level of the atrium.

Within the open-shunt group, the subgroup with persistent AF was compared with the subgroup with self-terminating AF. At death, mean LA pressure in goats with persistent AF was 26 ± 9 mm Hg versus 21 ± 2 mm Hg in goats with self-terminating AF (P = .096). Also, a trend toward a larger LA was found in the subgroup with persistent AF compared with that seen in the subgroup with self-terminating AF. More specifically, values were as follows: 126% ± 16% versus 117% ± 9% after 1 week (P = .42), 137% ± 17% versus 122% ± 13% after 2 weeks (P = .03), 142% ± 19% versus 126% ± 13% after 3 weeks (P = .15), and 144% ± 22% versus 122% ± 11% after 4 weeks (P = .07), respectively. The smaller number of animals might have lacked power to determine statistical significance.

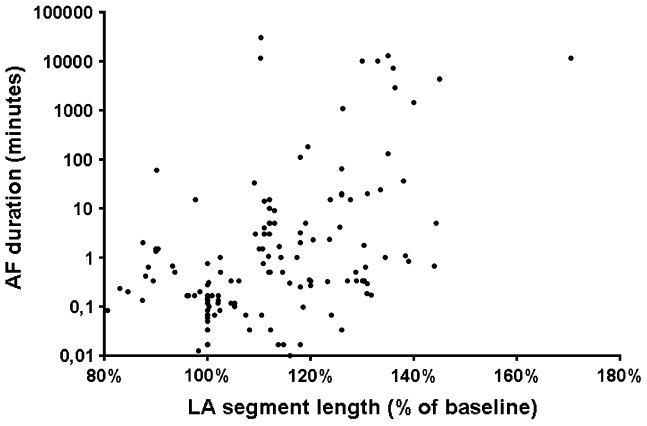

Figure 4. Correlation between atrial fibrillation *(AF)* duration and left atrial *(LA)* size. All AF registrations and their corresponding LA segment length are plotted. Longstanding AF could only be induced if LA segment length exceeded 110%.

Atrial Electrophysiology

In the open-shunt group the AERP increased from 151 ± 18 ms before the shunt to 178 ± 28 ms 1 week after shunt implantation (P = .01) and continued to be prolonged until death (Figure 5). In the control group the AERP did not change significantly. Compared with the control group, the increase in AERP was statistically significant in the third (P = .04) and fourth (P = .03) weeks.

There was no statistically significant difference between both groups concerning the conduction time during the chronic monitoring period (results not shown).

In the postoperative monitoring period AF episodes were recorded early after onset. The AF cycle length did not change significantly during chronic overload (first week, 149 ± 15 ms; fourth week, 136 ± 8 ms). However, a change in morphology of the atrial electrogram was observed in several animals. A typical example of a series of electrograms, registered early after onset of AF, in an open-shunt goat with spontaneous AF discontinuation is shown in Figure 6.

Histology

No change in the LA wall thickness was observed as a result of 4 weeks of overload (3.6 ± 1.3 mm in the open-shunt group vs 3.0 ± 1.5 mm in the control group). In the open-shunt group LA tissue slices stained with hematoxylin and eosin appeared normal. In one goat spots of myocyte necrosis and inflammatory cells enclosing the myocytes were observed. Structural changes in nuclear heterochromatin values, loss of contractile material, or other signs of degenerative transformation of cardiac myocytes could be noticed in

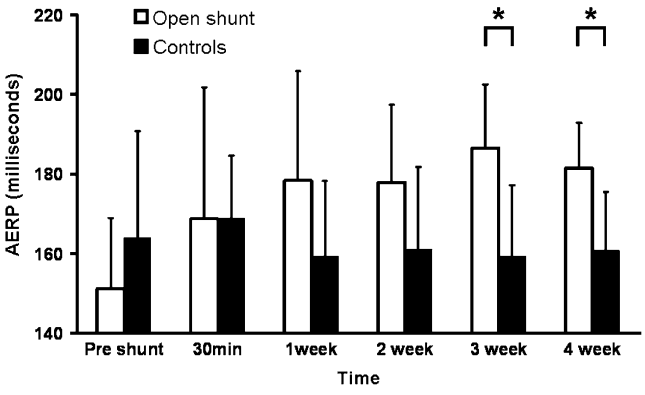

Figure 5. Operative and postoperative evolution of the atrial effective refractory period *(AERP)* in the open-shunt and control groups. In the open-shunt group an increase in AERP was noticed as a result of overload that became significant after 3 weeks *(*)*.

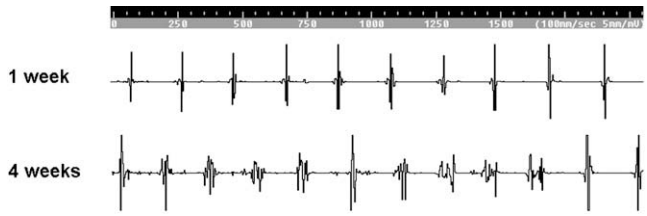

Figure 6. Bipolar atrial electrogram in an open-shunt goat. Atrial fibrillation registration early after its initiation and after 1 and 4 weeks of left atrial overload is shown. After 1 week, the electrogram presented a single steep negative deflection of high amplitude separated by an isoelectric segment. Several weeks of overload transformed the electrogram into more fragmented signals with groups of multiple deflections of unequal amplitudes.

none of the animals. Trichrome staining revealed an inflammatory reaction of the visceral pericardium in all goats, without differences between groups. In addition, a diffuse intensification of collagen in the atrial myocardium was noticed. However, after quantitative analysis, no significant differences could be demonstrated between groups (14% ± 6% in the open-shunt group vs 15% ± 5% in the control group). Furthermore, a marked regression in intracellular glycogen depots was noticed after 4 weeks of overload. Nevertheless, quantitative comparison showed no significant difference between the open-shunt group (7.7% ± 2.7%) and the control group (13.5% ± 4.9%, $P = .1$). Evaluation of left ventricular tissue samples after 4 weeks of overload showed normal myocyte structure with only little fibrotic accentuation.

Discussion

In this study the implantation of a polytetrafluorethylene vascular shunt between the aorta and the LA was feasible and reproducible. The shunt induced LA overload that was documented by an acute and chronic increase in LA pressure and volume. During a monitoring period of weeks, there was a significant increase in AF duration after burst pacing. In half of the animals, the longest observed episode of AF passed 1 week (persistent AF).

Clinical studies have shown that atrial dilatation is an important risk factor for AF. Grigionni and associates[16] studied the onset of AF in patients with mitral regurgitation initially in sinus rhythm. He concluded that age and LA dimension were independent predictive factors in the development of AF. Several animal models have been developed to mimic this clinical pathology. Yamauchi and associates[10,11] described a dog model of LA overload by using a shunt between the subclavian artery and a pulmonary vein. After 5 months with the shunt, animals were anesthetized, and atrial flutter or AF could be induced in 90% of the animals. In goats a chronic atrioventricular block resulted in a biatrial dilatation.[9] After 4 weeks, there was a slight increase in atrial diameter (+13.5% ± 3.9%), together with increases in AF duration to 6.4 minutes. The difference in LA dilatation between the atrioventricular block model and the current shunt model is the higher pressure and volume overload induced by the shunt. In a mitral regurgitation model partial disruption of the mitral chordae resulted in an abrupt atrial overload, with an increase in LA length of 30% after 4 weeks.[8] Animal losses were not mentioned. AF stability could only be evaluated once during general anesthesia in open-chest experiments. Half of the animals showed sustained AF (1 hour), and the others were nonresponders. The current shunt model produced a similar amount of dilatation (34.5% ± 20.9%). However, AF stability could be examined repeatedly and in nonanesthetized animals. AF exceeded 1 hour in 9 of 12 goats and persisted for more than 7 days in 6 animals. Moreover, overload was released progressively and could even be temporarily stopped by (partially) reclamping the shunt. All this resulted in a smoother LA overload and no operative deaths. An additional advantage of the present model is the possibility of shunt neutralization to investigate reversibility of overload.

The relationship between atrial dilatation and AF duration was examined. The correlation index was low, indicating no linear relation. However, in contrast to short AF durations that were inducible regardless of LA size, long AF durations (>100 minutes) were only obtained after a considerable increase in LA segment length (>110%). This might indicate a dilatation threshold necessary for longer AF durations. Altogether, these data show that factors other than dilatation might contribute to the increased AF duration in this model.

AF has been mentioned as a regular complication after cardiac interventions.[17] Prolonged AF could not be induced in the current control group, countering the inflammatory surgical response as a major causal factor. In animal studies and in human subjects, chronic heart failure has been forwarded as a promoter of AF.[18] The hemodynamic changes induced by the aorta-atrial shunt might finally lead to cardiac decompensation. However, 4 weeks of overload did not result in a significant increase in body weight or heart rate. Moreover, AF increased early after initiating overload, making heart failure a primary promoter of AF less likely in the present model. Regarding the multiple wavelet theory, the number of waves that can coexist in atria is determined by atrial tissue mass and wavelength, with wavelength being the product of refractory period and conduction velocity. A decrease in AERP was forwarded as a causal factor of AF in the rapid atrial pacing model.[19-21] In the current study an increase in AERP and an unchanged conduction velocity were observed. Similar results were presented in the mitral regurgitation model.[8] In the atrioventricular block model the AERP remained constant, with a slight increase in conduction velocity.[9] These findings show that the increase in AF duration in overload models is not related to a shortening of the refractory period.

In human subjects little information exists about histologic alterations in dilated atria and their possible relation to AF. Most of the literature describes the histologic consequences of AF. Frustaci and coworkers[22] studied patients with lone AF. Their findings were diverse, varying from myocarditis to noninflammatory cardiomyopathy and patchy fibrosis. Likewise, animal models were not uniform with regard to the impact of dilatation on atrial ultra structure. Some show increased interstitial fibrosis,[8] and others show no change.[9] In the present study, although a considerable amount of collagen was found in the atria, there was no significant difference between groups. Fibrosis could therefore not be identified as a causal factor for AF in the current model.

Thus alternative mechanisms responsible for AF need to be considered in dilatation models. Electrical uncoupling of cells as a result of stretch might induce a substrate for anatomically defined macroreentrant circuits, which might explain the high AF stability in the absence of AERP shortening. Furthermore, although spontaneous AF was not observed, an increase in ectopic atrial triggers responsible for the maintenance of AF could not be excluded. Also, spatial heterogeneities in conduction, as shown by Neuberger and associates,[9,23] might result in a substrate of AF in the absence of fibrosis and changes in the mean conduction velocity. Future studies with the presented model are planned to study these mechanisms in detail.

A limitation of the current model is that transthoracic echocardiographic screening is not reliable in the goat. Therefore chronic instrumentation with piezo-electric crystals through the skin is necessary to allow repetitive evaluations in nonanesthetized animals. However, the use of crystals on the LA lateral wall for dilatation measurement might pass by segmental dilatation in other regions of the atrium. Moreover, epicardial instrumentation and surgical intervention by itself resulted in a high amount of fibrosis (14%).

Another limitation is that intrathoracic vascular shunt construction remains a major operation in animals. In the literature animal losses are rarely mentioned.[8,9,20] In the current study 5 of 24 goats died, confirming the feasibility of the model.

Third, in human pathology LA dilatation initiates discreetly and develops slowly, and AF appears spontaneously in patients who have had overload for years. The overload in the present animal model is more abrupt. Smaller shunts and an extended follow-up period might overcome this inconvenience.

In conclusion, the current study demonstrates the feasibility of an animal model of LA overload induced by a shunt between the thoracic aorta and the LA. Increases in LA pressure and progressive dilatation of the atrium are documented. During chronic overload, prolonged and even persistent (>1 week) AF is inducible in most of the animals in the absence of a decreased AERP. Apart from LA size, no structural or ultrastructural changes can be forwarded as promoting factors for AF. Although dissimilarities remain, the current model exhibits resemblance with human pathology, in which AF is prevalent in dilated atria without shortening of atrial refractoriness. By using this model, future studies can be performed to investigate the responsible mechanisms for AF and the possible reversibility of dilatation and AF.

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